

## YEAST MODEL FOR STUDYING HERITABLE MAMMALIAN PRION DISEASE

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### Background and Objectives

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are infectious fatal neurodegenerative disorders that include a variety of human diseases, such as Creutzfeldt-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), kuru and fatal familial insomnia [1, 2]. Mammalian prion protein (PrP) in an abnormal aggregation-prone self-perpetuating (prion) conformation has been implicated as a TSE infectious agent. However, the mechanism of conversion of this protein into a prion conformation is poorly understood, thus preventing efficient development of both therapeutic interventions and prophylactic measures, targeting prion diseases. Although mutations leading to the heritable form of human prion diseases have been uncovered, primary effects triggering prion formation by mutant proteins were difficult to investigate due to the complexity of both experimental models and physiological effects of the disease. Proteins capable of producing the self-perpetuating (prion) conformations have also been identified in yeast [3, 4]. The power of yeast genetics and molecular biology has led to tremendous progress in understanding yeast prions. However, yeast prion proteins are not homologous to mammalian PrP. This prevented direct extrapolation of yeast data to mammalian and human prion diseases.

Our project utilized relatively cheap and powerful techniques of yeast genetics for studying prion properties of mammalian PrP. This enables us to uncouple the molecular events leading to a disease from their physiological consequences. Our approach allows molecular dissection of the prionogenic properties of mammalian PrP via genetic analysis in yeast, and paves the way for developing the new therapeutic or prophylactic treatments aimed at prion and amyloid disorders.

The overall goal of the project was to establish a yeast-based model for studying the molecular mechanism of prion formation by mammalian PrP protein. Our experimental assay is based on detection of the changes in the conformation and/or aggregation state of a mammalian protein via the subsequent heritable conformational change in a yeast protein, fused to it. Prion formation by yeast protein leads to an easily detectable phenotype, that is, growth on a certain selective medium. We introduced alterations, known to cause or prevent prion diseases in mammals or humans, into a PrP portion of the chimeric protein, and studied effects of these mutations on prion induction by chimeric protein in yeast. This tells us how and at which stages these mutations influence prion formation. New alterations affecting prion formation can also be identified by such an approach.

### Methods

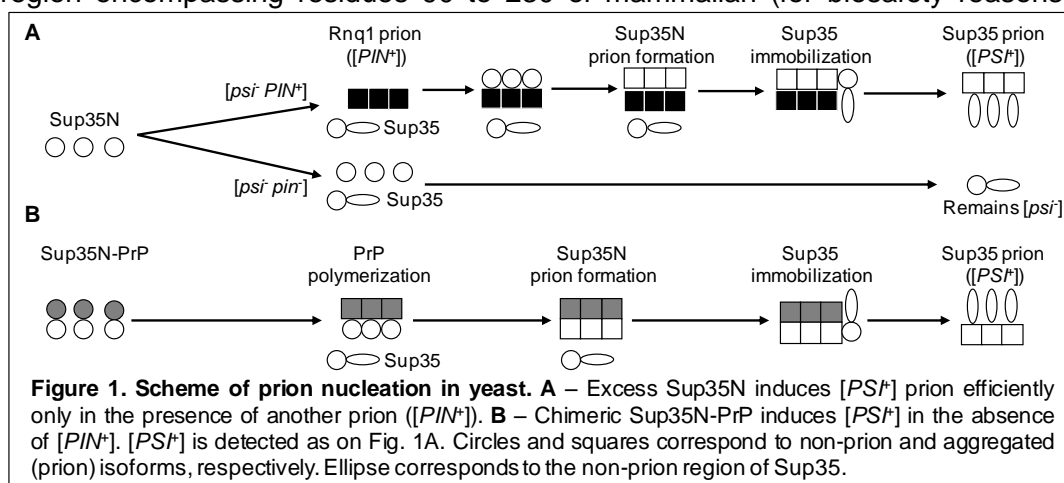
PrP prion produces cross- $\beta$  fibrous aggregates (amyloids) in the infected brains. The likely mechanism of prion formation is immobilization of the monomeric protein into amyloidogenic polymers, accompanied by conversion into the  $\beta$ -rich conformation [1, 2]. For heritable TSEs (including heritable forms of CJD), either prion formation or its propagation are promoted by a mutation in PrP [2, 5]. However, systematic studies of the mechanisms by which mutations influence a prion were difficult due to high complexity of the experimental models and disease-associated phenotypes. Sporadic prion diseases probably originate from spontaneous

nucleation of PrP polymers. However, due to low frequency and long incubation periods, this is essentially impossible to study in mammals.

Proteins that form self-perpetuating cross- $\beta$  polymers, similar to mammalian prions, have also been identified in yeast. One example is Sup35, a translation termination factor [3,4]. Its N-terminal region (Sup35N) is responsible for prion properties, while the C-terminal region functions in translation. Conversion of Sup35 into a prion results in the readthrough of stop codons, a phenotype that is easily detectable by growth in the specially designed yeast strains [3].

Our approach (Fig. 1) employs the chimeric construct (called N-PrP), in which yeast Sup35N is fused to the region encompassing residues 90 to 230 of mammalian (for biosafety reasons, mouse) PrP (PrP<sub>90-230</sub>).

PrP<sub>90-230</sub> is able to form  $\beta$ -rich aggregates, and is sufficient to maintain the infectious prion state in mammals [2, 5]. We have shown that



introduction of N-PrP into a yeast cell greatly increases efficiency of the initial nucleation of Sup35 prion in the absence of any pre-existing prions. Newly nucleated prion incorporates full-size Sup35, allowing detection by growth assay (Fig. 1B). Thus, PrP-promoted nucleation is fixed in the form of a readily detectable yeast prion. In such a design, amyloidogenic properties of a mammalian protein can be monitored via prion formation by the yeast protein, that is easily detectable at phenotypic level. Biochemical (centrifugation and detergent insolubility) and cytological (secondary immunofluorescence) approaches were used to confirm N-PrP aggregation in yeast.

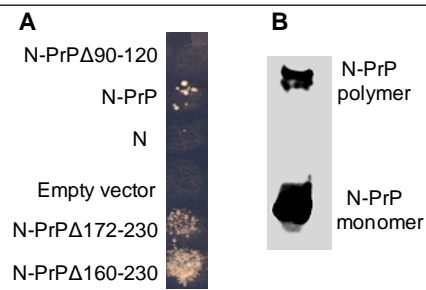
## Results

Our preliminary data, showing that N-PrP promotes de novo prion formation in yeast in the absence of any pre-existing prions, have been reproduced by using constructs under two different inducible promoters, namely the copper-inducible promoter (as shown on Fig. 2A) and galactose-inducible promoter (not shown). This result confirms that observed effects are not related to chemicals that were used to induce N-PrP expression in yeast.

Our preliminary data showing that deletion of the PrP residues 90-120, knocking out prion propagation in mammals [2, 5], also antagonizes prion nucleation in yeast, have been confirmed (Fig. 2A). We have also demonstrated that deletion 90-120 does not influence the levels of the N-PrP protein, indicating that this deletion influences the ability to produce a prion, rather than protein production.

We have shown that N-PrP constructs with the deletions of the C-terminal region (after amino acid positions 160 or 172) promote de novo prion formation in yeast with the significantly higher

**Figure 2. Effects of PrP on prion nucleation in yeast.** **A** – PrP deletion  $\Delta 90-120$ , eliminating susceptibility to prion disease in mammals, abolishes prion nucleation in yeast, while C-terminal deletions  $\Delta 160-230$  and  $\Delta 172-230$  increase prion nucleation. **B** – Small fraction of polymerized N-PrP is detected on the top of the gel in the *[psi<sup>+</sup> pin<sup>-</sup>]* strain after overproduction.



frequency, compared to the original N-PrP construct (Fig. 2A). Nonsense-mutation at position 160 of human PrP, generating a protein that is similar to one of our C-terminal deletions ( $\Delta 160$ ), is associated with the heritable form of prion disease in human [6]. The other deletion ( $\Delta 172$ ) represents a new alteration whose effect was not known previously. Increased promotion of prion formation occurs despite the fact that the same deletions decrease rather than increase levels of N-PrP protein produced in yeast. The most plausible explanation is that C-terminal deletions destabilize the N-PrP construct, leading to both increased degradation and increased aggregation (followed by prion formation). This agrees with some models of mammalian PrP structure.

We have shown that while most of the N-PrP protein accumulated in the absence of pre-existing prions is soluble in detergents, N-PrP overproduction leads to formation of the small but detectable detergent-resistant polymeric fraction (Fig. 2B) as expected of an amyloid. N-PrP overproduction also promotes formation of filamentous cytologically detectable aggregates, associated with de novo prion formation [7,8], by GFP-tagged Sup35. This suggests that promotion of de novo prion formation by N-PrP occurs through generation of the transient aggregate structures.

Prions exist in multiple variants or “strains” [1-4]. Even if most of the N-PrP prions, initially formed in yeast, are transient and unstable, some rare variants capable of stable propagation might appear. To identify them, we have transiently overexpress chaperone Hsp104 in the *[PSI<sup>+</sup>]* isolates, induced in the presence of overproduced N-PrP. Excess Hsp104 cures *[PSI<sup>+</sup>]* but not other prions [3,9,10]. Resulting *[psi<sup>-</sup>]* colonies (retaining N-PrP) were screened for de novo *[PSI<sup>+</sup>]* nucleation at normal levels of N-PrP (without overproduction). Dependence of the “nucleation” phenotype of N-PrP and absence of the other known prions was confirmed by genetic and biochemical assays. One N-PrP isolate capable of inducing Sup35 prion without overproduction was identified. It is curable by guanidine hydrochloride, as expected of a yeast prion. As per our model, this isolate should contain a prion form of N-PrP controlled through its PrP portion and capable of propagating in yeast.

## Conclusions

1. New assay for studying the prion properties of mammalian prion protein in yeast is developed. This assay is based on the ability of a chimeric protein, composed of the fragments from yeast prion protein Sup35 and mammalian PrP, to nucleate de novo formation of the yeast prion in the absence of any known pre-existing prions.
2. The region of mammalian PrP protein encompassing the amino acid positions 90 through 120, that is required for prion propagation in mammals, is also essential for prion formation in yeast.

3. Deletion of the C-terminus of PrP protein after amino acid position 160, resembling a form generated in one of the heritable prion diseases in humans, also increases de novo prion formation in yeast.
4. New PrP alteration promoting de novo prion formation (deletion of the C-terminus after position 172) is detected by using yeast assay.
5. Prion induction in yeast is accompanied by the formation of the detergent-insoluble fraction of a chimeric protein.
6. An isolate of N-PrP that is capable of propagating the prion-like state in yeast has been identified.

## References

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Yury O. Chernoff  
Professor, School of Biology, Georgia Institute of Technology  
310 Ferst Drive, M/C 0230, Atlanta, GA 30332-0230  
Tel. 404-894-1157, fax 404-894-0519, E-mail yury.chernoff@biology.gatech.edu